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Responses of 'Fuji' apples to short and long duration exposure to elevated CO_2 concentration

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Abstract

Fruit maturity, diphenylamine (DPA) treatment and controlled atmosphere (CA) storage regimes were evaluated as factors affecting development of 'Fuji' apple CO_2 injury. The incidence and severity of CO_2 injury (brown-heart) increased with advanced maturity and greater watercore severity at harvest. The development of CO_2 injury during storage was prevented by DPA treatment. The severity of CO_2 injury was higher in fruit exposed to 20 kPa CO_2 after harvest than in fruit exposed to 20 kPa CO_2 after 8 months of CA storage. Ethanol, acetaldehyde and methanol concentrations increased during short- and long-term exposure to high CO_2, however, DPA reduced ethanol and acetaldehyde accumulation. Storage in low O_2 (0.5 kPa O_2, 0.05 kPa CO_2) also stimulated accumulation of ethanol, acetaldehyde and methanol however, no internal disorders developed with or without the use of DPA. The harvest date effect on severity of CO_2-injury following short-term exposure to 20 kPa CO_2 was related to severity of CO_2-injury after long-term CA storage with 3 kPa CO_2.

Keywords: Fruit maturity; Brown heart; CA storage; Acetaldehyde; Ethanol; Methanol

1. Introduction

'Fuji' apples are tolerant to low O_2 partial pressure when CO_2 partial pressure is low (Fan, 1992; Argenta et al., 1994). However, a high incidence of low O_2 injury has been reported for CA stored fruit (Park and Lee, 1991; Park et al., 1997). 'Fuji' apples can develop core-browning and core-line browning during storage. Core-line browning may be related to the presence of watercore (Fukuda, 1984), while core-browning is a senescent disorder (Fan, 1992). 'Fuji' apples can also develop CO_2-induced cortex browning, brown-heart, during CA storage (Park and Lee, 1991; Argenta et al., 1994; Fan et al., 1997). High CO_2 and low O_2 injury can be induced during short-term exposures to high CO_2 and/or low O_2 at ambient temperature (Ke et al., 1991). Volz et al. (1998) reported that short-term exposure of 'Fuji' apples to 20 kPa CO_2 at harvest was a more reliable method for predicting susceptibility to CA-induced cortex browning than maturity indices. The variation in apple fruit susceptibility to

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CO_2 injury is an important factor in determination of optimum CA regimes for 'Fuji' apple storage.

The risk of CO_2-related cortex browning in many apple cultivars increases when rapid CA storage procedures are utilized and when the antioxidant diphenylamine (DPA) is not used for control of superficial scald (Watkins et al., 1997a). Pre-storage treatment with DPA reduces cortex browning in 'Delicious' apples (Meheriuk et al., 1984) and CO_2-induced injuries in many apple cultivars (Burmeister and Dilley, 1995; Watkins et al., 1997b; Colgan et al., 1999).

The mechanism by which CO_2 causes injury in fruit cells is not clearly understood. Accumulation of succinate in 'Bramley's Seedling' apples exposed to 15 kPa CO_2 may lead to the development of brown-heart (Hulme, 1956). Acetaldehyde and ethanol concentrations increase when apple fruit are exposed to reduced O_2 or elevated CO_2 concentrations and cortex browning can be simulated by injection of acetaldehyde or ethanol into 'Delicious' and 'Jonathan' apples (Clijsters, 1965; Smagula et al., 1968). Choi (1997) suggested that browning in 'Fuji' apples stored in 3 kPa CO_2 is associated with increased membrane permeability, decompartmentation and oxidation of phenolic compounds.

The objective of the present study was to determine susceptibility of 'Fuji' apples to CO_2-injury as influenced by harvest maturity and DPA treatment at harvest. The incidence of CO_2-injury induced by short-term, 20 kPa CO_2-storage at 20 °C was compared to injury observed after long-term CA cold storage. The possible relationship between internal disorders and accumulation of fermentative compounds was also evaluated.

2. Materials and methods

Fruit of *Malus domestica* Borkh cv. Fuji were harvested 162, 173 and 188 days after full bloom (DAFB) from two commercial orchards near Wenatchee, WA, in 1997. Treatments with DPA were applied the day of harvest by submerging apples for 1 min in 2000 μl l^{-1} DPA using a formulated Shield-DPA 15% emulsion (Shield-Brite Corp., Kirkland, WA).

To assess responses to short-term exposure to 20 kPa CO_2, apples were enclosed in 76 μm thick plastic bags 24 h after harvest. Bags were purged at 6 l h^{-1} with compressed air or a mixture of air and compressed CO_2 (10 kPa O_2, 20 kPa CO_2). Fruit were held at 20 °C for 3, 6, 9 or 12 days. The O_2 and CO_2 concentrations were monitored by electrochemical and infrared gas analyzers (California Analytical Instruments, Orange, CA), respectively.

For long-term storage, fruit were cooled overnight at 1 °C, then enclosed in 0.145 m^3 chambers and stored 4 or 8 months at 0.5 kPa O_2 + 0.05 kPa CO_2 or 1.5 kPa O_2 + 3 kPa CO_2. The O_2 concentration was reduced beginning 36 h after harvest. Storage chamber atmospheres were established within 60 h after harvest and monitored at 90-min intervals (Techni-Systems, Chelan, WA). Semi-static chamber atmospheres (purged only when atmosphere adjusted) were maintained with N_2 generated from a membrane system (Permea, St. Louis, MO), compressed air and CO_2. The low (≤ 0.05 kPa) CO_2 concentration was maintained by adding 0.1 kg of hydrated lime ($Ca(OH)_2$) per kilogram of fruit.

Additionally, fruit harvested 188 DAFB were stored in 1.5 kPa O_2 + 0.05 kPa CO_2 at 1 °C. After 8 months, apples were removed from storage, held overnight at 20 °C, then exposed to 10 kPa O_2 + 20 kPa CO_2 for 12 days. There were 18 single fruit replicates for each orchard × harvest date × storage treatment × storage duration combination.

Maturity of 18 individual apples was determined 24 h after each harvest date by analyses of respiration, internal ethylene concentration (IEC), peel color, firmness, titratable acidity (TA) and estimation of starch scores. Firmness was measured on two pared surfaces per fruit using a penetrometer with an 11 mm tip (Lake City Technical, Kelowna, BC, Canada). TA was determined by titrating 10 ml of juice with 0.1 M KOH to pH 8.2 using an autotitrator (Radiometer, Copenhagen, Denmark). The extent of starch hydrolysis (SI) was rated visually using a 1–6 scale (1 = full, 6 = no starch) after staining an equatorial section of each apple with a 5 mg l^{-1} I-KI solution. For respiration analyses, four replicates of six apples

each were enclosed in 20 l chambers maintained at 20 °C and supplied with compressed, ethylene-free air at 100 ml min^{-1}. Effluent air was analyzed for CO_2 concentration by a gas chromatograph (HP 5890; Hewlett–Packard, Palo Alto, CA) equipped with a methanizer (John T. Booker, Austin, TX), flame ionization detector and a 0.6 m, 2 mm i.d. stainless steel column packed with 80–100 mesh Porapak Q (Supelco, Bellefonte, PA). Oven, detector, methanizer and injection temperatures were 50, 200, 290 and 150 °C, respectively. Gas flows for N_2, H_2 and air were 70, 30 and 300 ml min^{-1}, respectively. Production of CO_2 by fruit previously stored was analyzed 36 and 24 h after removing fruit from short-or long-term storage, respectively. Internal ethylene, acetaldehyde, ethanol and methanol concentrations of individual apples were measured in gas samples removed from the fruit core (Williams and Patterson, 1962). Analyses of internal ethylene, acetaldehyde, ethanol and methanol were performed using a gas chromatograph (HP 5890; Hewlett–Packard) equipped with a flame ionization detector and a 0.5 m, 3.2 mm i.d., glass column packed with 80–100 mesh Porapak Q (Supelco, Bellefonte, PA). Oven, detector and injection temperatures were 90, 200 and 100 °C, respectively. N_2, H_2 and air flows were 25, 25 and 300 ml min^{-1}, respectively. A standard gas mixture containing 8.7 mmol m^{-3} ethanol, 9.1 mmol m^{-3} acetaldehyde and 12.6 mmol m^{-3} methanol was prepared by injecting 5 μl of a standard solution containing 0.29 M ethanol, 0.28 M acetaldehyde and 0.20 M methanol in hexane into a 2 l gas dilution bottle previously purged with helium. The solution was evaporated with stirring for 30 min at 25 °C. After the equilibration period, a 0.5 ml gas sample was withdrawn from the dilution bottle and analyzed by GC. Confirmation of methanol in the fruit was by GC-MS (HP 5890, HP5971; Hewlett–Packard) and the Wiley NBS library, as described previously (Mattheis et al., 1998).

Watercore and CO_2-injury were visually scored on fruit cut through the equator. Watercore severity was rated using a scale from 1 = no watercore to 5 = very severe watercore. The severity of CO_2-injury (brown-heart) was scored as: (1) clear; (2) 1–30%; (3) 31–60%; or (4) $>60\%$ of cortex dark brown.

Data analyses were performed using the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Treatment effects were analyzed by the ANOVA procedure and treatment mean separation was determined by Fischer's LSD or Duncan's multiple range tests ($P \leq 0.05$). The relationships between CO_2-injury and core volatile concentrations were examined using the Kendall's tau-b coefficient. No significant effects of the two orchards were present in the study of maturity and DPA effects on CO_2-injury, therefore the data from both orchards was combined and 36 single fruit replicates per treatment were used.

3. Results

3.1. Maturity at harvest

Respiration rate, internal ethylene concentration (IEC), starch index (SI) and severity of watercore (WC) increased, whereas firmness and titratable acidity (TA) decreased between 162 and 188 DAFB (Table 1).

Table 1
Respiration rate, internal ethylene concentration (IEC), starch index (SI), firmness, titratable acidity (TA) and watercore (WC) severity of 'Fuji' apples at harvest[a]

Harvest date[b]	CO_2 evolution (μmol kg^{-1} h^{-1})	IEC μmol m^{-3}	SI (1–6)	Firmness (N)	TA (%)	WC (1–4)
162	367	46	3.3	82	0.40	1.9
173	431	107	4.3	78	0.36	2.9
188	496	240	5.4	75	0.32	3.4
$LSD_{0.05}$	41	53	1.0	2.1	0.03	0.6

[a] Data from two orchards are combined.
[b] Days after full bloom.

Table 2
Severity of CO_2-injury (brown-heart), respiration rate, internal concentrations of ethanol, acetaldehyde and methanol in 'Fuji' apples harvested 162, 173 and 188 days after full bloom (DAFB) and stored in CA at 0.5 °C with 0.5 or 1.5 kPa O_2 and 0.05 or 3 kPa CO_2 for 4 or 8 months[a]

Treatments p_{O_2}: p_{CO_2}[b] (kPa)	4 months					8 months				
	CO_2 injury (1–4)	CO_2 evolution (μmol kg^{-1} h^{-1})	Ethanol (mmol m^{-3})	Acetaldehyde (μmol m^{-3})	Methanol (μmol m^{-3})	CO_2 injury (1–4)	CO_2 evolution (μmol kg^{-1} h^{-1})	Ethanol (mmol m^{-3})	Acetaldehyde (μmol m^{-3})	Methanol (μmol m^{-3})
162 DAFB										
0.5: 0.05	1.00b	288a	0.75b	139b	0.61b	1.00b	378a	0.18a	181b	9.75a
0.5: 0.05 +DPA	1.00b	263a	0.31c	66c	0.11c	1.00b	271b	0.06b	276a	5.00b
1.5: 3	1.16a	255a	1.27a	298a	1.85a	1.27a	374a	0.14b	131c	4.80b
1.5: 3+DPA	1.00b	236a	0.40bc	158b	0.55b	1.00b	316ab	0.05a	121c	5.30b
173 DAFB										
0.5: 0.05	1.00b	390a	2.32a	560a	2.59a	1.00b	352a	1.11ab	511a	11.25a
0.5: 0.05 +DPA	1.00b	306b	1.28b	421b	1.55b	1.00b	257b	1.40ab	508a	8.55b
1.5: 3	1.61a	325b	1.67ab	516a	1.89ab	1.33a	370a	1.64a	307b	5.60c
1.5: 3+DPA	1.00b	312b	0.37c	231c	0.24c	1.00b	292ab	0.90b	154c	6.85bc
188 DAFB										
0.5: 0.05	1.00b	462a	4.86a	804a	5.17a	1.00c	509a	4.24a	583a	12.90a
0.5: 0.05 +DPA	1.00b	355b	3.82b	527b	4.01b	1.00c	402b	1.63bc	436b	3.45c
1.5: 3	2.27a	408ab	2.30c	579b	2.16c	1.83a	468ab	2.37b	351c	8.70b
1.5: 3+DPA	1.00b	364b	1.12d	243c	0.93d	1.06b	417b	0.95c	352c	3.15c
Significance										
Harvest (H)	***	***	***	***	***	***	***	***	***	***
H × Treatment (T)	NS	*	*	*	**	NS	NS	*	***	*

[a] DPA treatment consisted of 2 min dip in 2000 μl l^{-1} DPA solution. Means with same letter for each storage period and harvest date are not significantly different (Duncan's multiple range test, $P \leq 0.05$).

[b] Partial pressure of O_2 and CO_2.

NS Nonsignificant.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

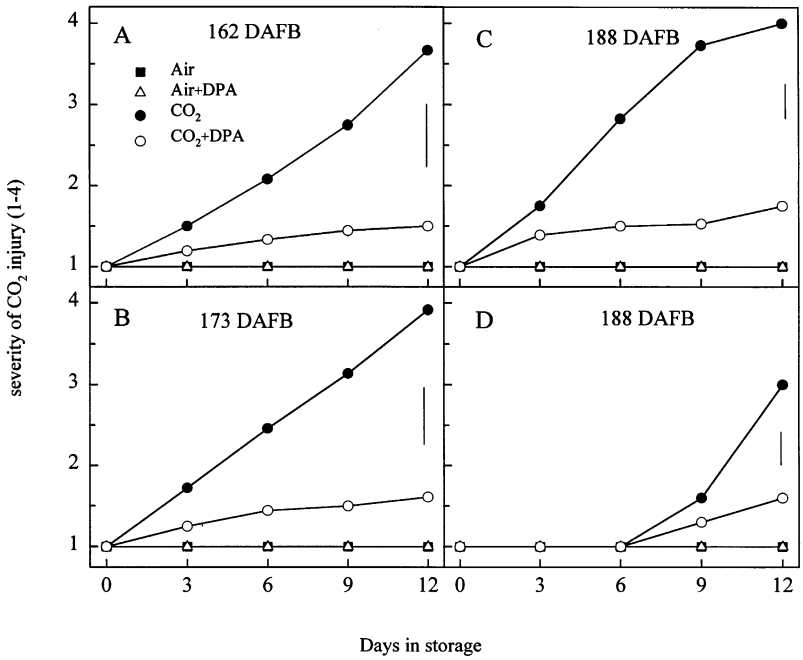

Fig. 1. Severity of CO_2-injury (brown-heart) in 'Fuji' apples harvested 162–188 days after full bloom (DAFB), stored at 20 °C in air or 10 kPa O_2 + 20 kPa CO_2 1 day after harvest (A–C) or after 8 months of low-CO_2 CA storage (D). Fruit DPA treatment consisted of 2 min dip in 2000 μl l^{-1} DPA solution. Data from two orchards are combined. Vertical bars represent $LSD_{0.05}$ for significant treatment × days interaction.

3.2. Effects of CO_2 exposure, fruit maturity and DPA treatment on development of CO_2-injury

The severity of CO_2-injury (brown-heart) increased with the duration of 20 kPa CO_2 exposure regardless of harvest date (Fig. 1). After 6 and 9 days of CO_2 exposure, severity of brown-heart was greater in fruit harvested 188 DAFB than 162 DAFB (Fig. 1A,C). In fruit exposed to 20 kPa CO_2 following 8 months of 0.05 kPa CO_2-CA storage (Fig. 1D), CO_2-injury appeared after 9 days and was less than that observed in fruit exposed to 20 kPa CO_2 at harvest.

Brown-heart after 4 and 8 months cold storage in 1.5 kPa O_2 + 3 kPa CO_2 (Table 2) was similar to that observed in fruit held for 3–6 days in 20 kPa CO_2 at harvest (Fig. 1). The severity of CO_2-injury developing during CA storage also increased with harvest date but did not change between 4 and 8 months storage (Table 2). The development of CO_2-injury was prevented by DPA treatment in fruit exposed to 20 kPa CO_2 for 3–12 days (Fig. 1) and in fruit stored 4 or 8 months in 3 kPa CO_2-CA (Table 2).

3.3. Effects of CO_2 exposure and DPA treatment on fruit respiration and production of ethylene and fermentative compounds

Respiration rate of fruit exposed to 20 kPa CO_2 without DPA treatment was higher than fruit stored in air (Fig. 2). For example, respiration rate after 9 days 20 kPa CO_2 exposure was 17, 20 and 23% higher for fruit harvested 162, 173 and 188 DAFB, respectively, compared with fruit stored in air. DPA treatment prevented the CO_2-induced increase in respiration rate. Fruit harvested 162 DAFB and treated with DPA had the lowest respiration rate following exposure to 20

kPa CO_2. For air-stored fruit, DPA had no effect on respiration rate. IEC was reduced by the 20 kPa CO_2 and DPA treatments.

The respiration rate of fruit without DPA stored 4 or 8 months in 0.5 kPa $O_2 + 0.05$ kPa CO_2 was similar to or greater than fruit stored in 1.5 kPa $O_2 + 3$ kPa CO_2 (Table 2). DPA treatment reduced respiration of apples stored in 0.5 kPa $O_2 + 0.05$ kPa CO_2 except for fruit harvested 162 DAFB and stored 4 months. However, DPA treatment did not significantly reduce respiration of fruit stored in 1.5 kPa $O_2 + 3$ kPa CO_2.

Increased CO_2 evolution following exposure to 20 kPa CO_2 was accompanied by increased ethanol, acetaldehyde (Fig. 3) and methanol (Fig. 4) concentrations in the fruit core. Depending on the harvest date and number of days in storage, DPA treatment reduced the CO_2-induced accumulation of ethanol and acetaldehyde, but had less effect on methanol.

Concentrations of ethanol, acetaldehyde and methanol in fruit without DPA stored in 0.5 kPa $O_2 + 0.05\%$ kPa CO_2 were similar to or higher than the concentrations in fruit without DPA

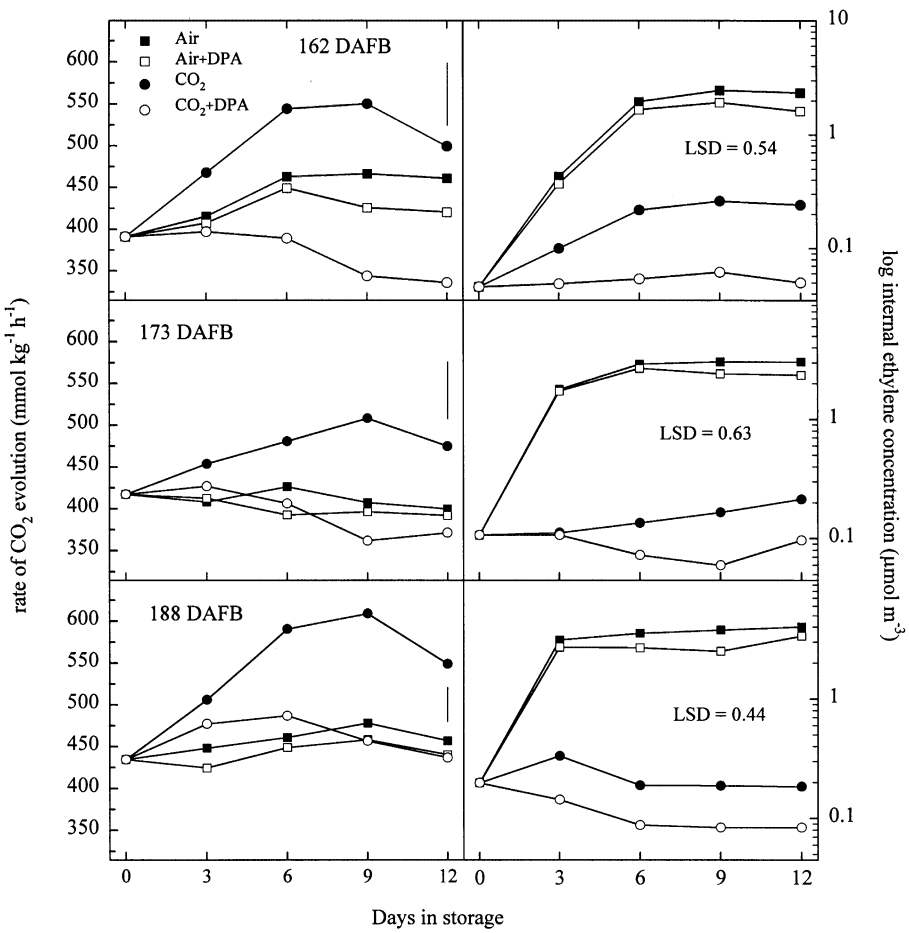

Fig. 2. Respiration rate and internal ethylene concentration of 'Fuji' apples harvested 162–188 days after full bloom (DAFB), stored at 20 °C in air or 10 kPa $O_2 + 20$ kPa CO_2 1 day after harvest. Initial gas analyses were conducted 36 h after removal from storage. DPA treatment consisted of 2 min dip in 2000 µl l^{-1} DPA solution. Data from two orchards are combined. Vertical bars represent $LSD_{0.05}$ for significant treatment × days interaction.

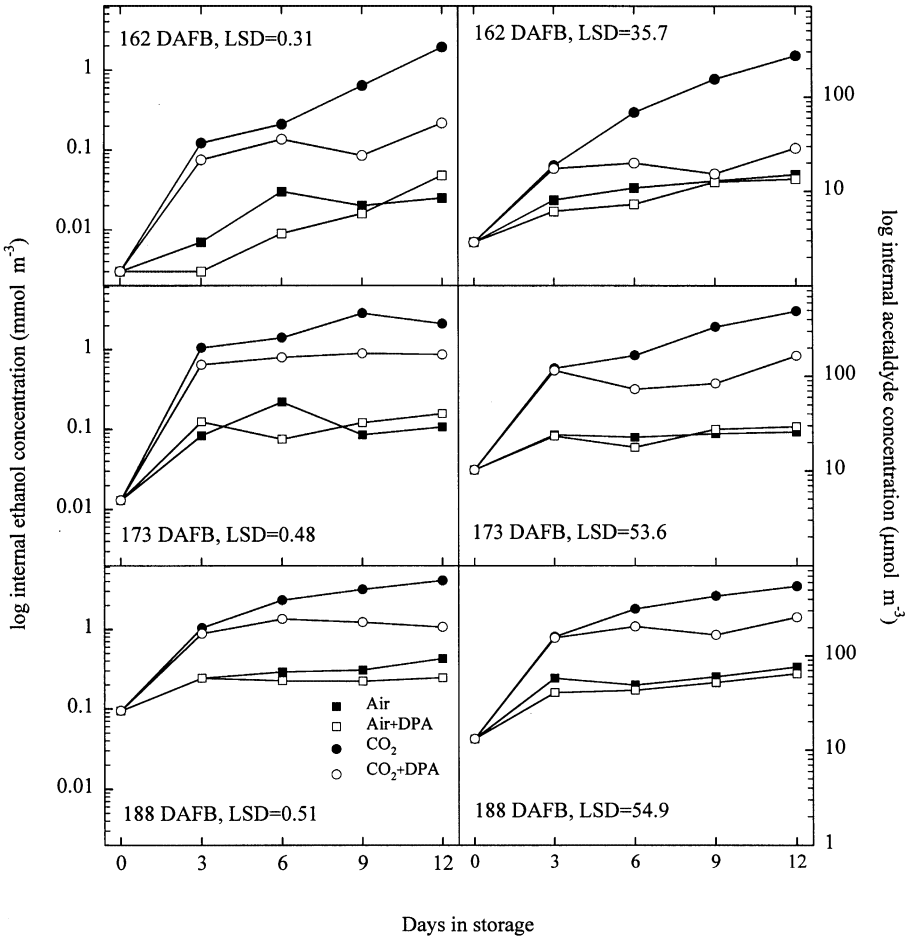

Fig. 3. Internal concentrations of ethanol and acetaldehyde of 'Fuji' apples harvested 162–188 days after full bloom (DAFB), stored at 20 °C in air or 10 kPa O_2 + 20 kPa CO_2 1 day after harvest. Initial gas analyses were conducted 36 h after removal from storage. DPA treatment consisted of 2 min dip in 2000 μl l^{-1} DPA solution. Data from two orchards are combined.

stored in 1.5 kPa O_2 + 3 kPa CO_2 (Table 2), except for apples harvested 162 DAFB and stored 4 months. DPA treatment resulted in similar or lower concentrations of ethanol, acetaldehyde and methanol than in untreated fruit after 4 months storage (Table 2). After 8 months storage, DPA effects were similar to those at 4 months except for fruit harvested 162 DAFB, where DPA treatment resulted in greater ethanol and acetaldehyde accumulation.

3.4. Correlations between CO_2-injury, watercore and accumulation of fermentative compounds

Correlations between CO_2-injury and watercore were significant for fruit harvested 173 or 188 DAFB and held for 9 days at 20 °C in 20 kPa CO_2 (Table 3). Correlations between CO_2-injury and ethanol, acetaldehyde and methanol for fruit held for 9 days at 20 °C in 20 kPa CO_2 were low but statistically significant. Correlations between CO_2-injury and watercore and concentration of fermentative volatiles for fruit held for 6 days in 20 kPa CO_2 were similar to that for fruit exposed for 9 days at the same temperature and atmosphere, but were not significant for fruit held for 3 or 12 days (data not presented). For fruit harvested 173 and 188 DAFB, CO_2-injury was correlated to watercore after 4 months CA storage (Table 3). CO_2-injury was less correlated or not

significantly correlated with ethanol and methanol after 4 months storage in 1.5 kPa $O_2 + 3$ kPa CO_2 than after short-term storage in 20 kPa CO_2.

4. Discussion

In this study, 'Fuji' apples stored in 1.5 kPa $O_2 + 3$ kPa CO_2 developed internal browning (brown-heart), while fruit stored in 0.5 kPa $O_2 +$ 0.05 kPa CO_2 or air did not (Table 2). Development of cortex browning and cavitation in 'Fuji' apples during storage in high CO_2 partial pressure, from 1 to 5 kPa, has been observed previously (Park and Lee, 1991; Argenta et al., 1994; Fan et al., 1997; Volz et al., 1998). Previous studies indicated that CO_2-induced peel injury is likely to be more severe in early harvested fruit (Meheriuk, 1977), while internal CO_2-injury is more likely to develop in apples harvested at a more advanced stage of maturity (Fidler et al., 1973; Meheriuk, 1977; Volz et al., 1998; Elgar et al., 1999). The greater incidence and severity of CO_2-injury in late harvest fruit observed in the present study may be related in part to higher respiration rate and watercore severity at the later harvest dates (Table 1). For individual fruit harvested 173 DAFB (commercial maturity) or 188 DAFB, CO_2-injury was correlated to watercore after 4 months CA storage (Table 3). Depending on the orchard, more mature, watercored 'Fuji' apples have a relatively low intercellular air space volume, resulting in a significant reduction of permeance to gas diffusion and an increase in internal CO_2 partial pressure (Argenta et al., 2001). Variability in development of CO_2 and O_2-related injury has been associated with differences in peel gas permeance and fruit respiration rate that may result in elevated CO_2 and reduced O_2 internal partial pressures (Dadzie et al., 1993; Park et al., 1993; Colgan et al., 1999; Elgar et al., 1999). Fukuda (1984) reported an association between watercore intensity in 'Fuji' apples at harvest and incidence of core-line browning after storage. However, Park et al. (1997) and Volz et al. (1998) did not find a significant correlation between severity of watercore at harvest and CO_2-injury after storage, indicating other factors may influence development of CO_2-injury besides harvest maturity and severity of watercore. Season and orchard may affect susceptibility of apple fruit to CO_2-injury. The incidence of CO_2-induced injury in 'Braeburn' apples, for example, is higher in fruit from colder regions or seasons, higher altitude districts and light cropping trees (Lau, 1998; Elgar et al., 1999).

Fruit sensitivity to CO_2-injury increased with advanced maturity (Fig. 1A,B; Table 2) but decreased during long-term storage (Fig. 1D). The severity of CO_2-injury during CA storage did not

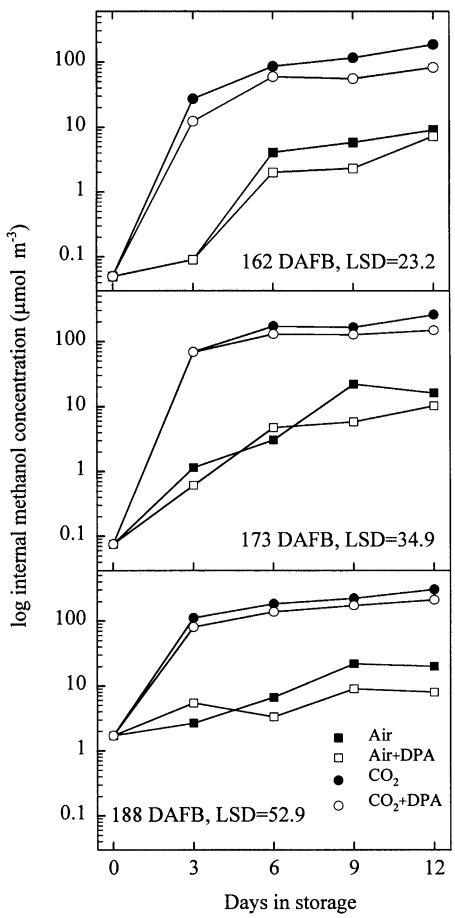

Fig. 4. Internal concentrations of methanol of 'Fuji' apples harvested 162–188 days after full bloom (DAFB), stored at 20 °C in air or 10 kPa $O_2 + 20$ kPa CO_2 1 day after harvest. Initial gas analyses were conducted 36 h after removal from storage. DPA treatment consisted of 2 min dip in 2000 μl l^{-1} DPA solution. Data from two orchards are combined.

Table 3
Kendall Tau b correlation coefficients between CO_2-injury (brown-heart) and watercore severity, and internal ethanol, acetaldehyde and methanol concentrations[a]

	After 9 days at 20 °C with 10 kPa O_2 + 20 kPa CO_2			After 4 months at 0.5 °C, 1.5 kPa O_2 + 3 kPa CO_2		
Harvest date[b]	162	173	188	162	173	188
CO_2 injury × watercore	0.32^{NS}	0.55*	0.47*	0.42^{NS}	0.63**	0.72***
CO_2 injury × ethanol	0.54***	0.53**	0.43**	0.44***	0.28^{NS}	0.27^{NS}
CO_2 injury × acetaldehyde	0.66***	0.49***	0.48***	0.56***	0.37***	0.32**
CO_2 injury × methanol	0.52***	0.43**	0.32**	0.19^{NS}	0.16^{NS}	0.29*

[a] Fruit were stored for 9 days at 20 °C or 4 months in CA.
[b] Days after full bloom.
^{NS} Nonsignificant.
* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

change significantly between 4 and 8 months storage (Table 2). These results indicate the susceptibility of 'Fuji' apples to CO_2-injury is highest during the initial weeks in storage after harvest and confirm our previous report (Argenta et al., 2000).

Fruit maturity at harvest and DPA treatment contribute to variation in severity of CO_2-injury after 20 kPa CO_2-exposure and are related to variation in severity of CO_2-injury after long-term CA storage. A high correlation between severity of CO_2-injury after short- and long-term storage was reported previously by Volz et al. (1998).

Treatment of 'Fuji' apples with DPA prior to storage suppresses the development of CO_2-injury (Fig. 1; Table 2). Depending on harvest date, DPA treatment not only prevented the development of CO_2-injury, but also slowed fruit ripening by reducing the respiration rate and IEC during long-term storage. DPA prevents development of apple superficial scald (Smock, 1957), internal browning and brown-heart (Meheriuk et al., 1984; Burmeister and Roughan, 1997; Colgan et al., 1999) and can reduce the rate of apple fruit ripening (Lurie et al., 1989). Unfavorable CO_2 concentrations disrupt aerobic respiration (Figs. 3 and 4) and have been demonstrated to reduce the concentration of the endogenous ascorbate (Bangerth, 1977). The antioxidant DPA prevents the development of CO_2-injury, indicating the development of CO_2-injury may be related to oxidative stress due to activation of oxidative processes or reduction of fruit antioxidant systems, as suggested by Watkins et al. (1997a).

Harvest maturity, concentration of CO_2 and O_2 during storage, storage duration and DPA treatment influence severity of CO_2-injury and accumulation of ethanol, acetaldehyde and methanol in 'Fuji' apples. The increase in CO_2 evolution associated with ethanol and acetaldehyde accumulation indicated a change to anaerobic metabolism after fruit were exposed to 20 kPa CO_2.

Ethanol and acetaldehyde accumulate in many fruit during ripening even under aerobic conditions (Fidler, 1968) and are precursors of natural aroma compounds (Knee and Hatfield, 1981). Ethanol is present in most apple cultivars in relatively small amounts, but may accumulate in overripe and senescent fruit (Fidler et al., 1973; Nichols and Patterson, 1987). Increased metabolic rate and malic enzyme activity associated with the climacteric rise in respiration could account for the ethanol and acetaldehyde increase in late harvested fruit (Neal and Hulme, 1958). In 'Fuji' apples, ethanol, acetaldehyde and methanol accumulated during ripening in air at 20 °C (Figs. 3 and 4). Concentrations of these compounds in the fruit also increased during the first 4 months of CA storage (data not presented), however, only acetaldehyde concentration was consistently cor-

related with CO_2 injury (Table 3). The lack of a significant correlation between ethanol concentration and CO_2 injury may have been due to increased ethanol present in fruit harvested 173 or 188 DAFB. Between 4 and 8 months CA storage, methanol concentration increased while concentrations of ethanol and acetaldehyde decreased, did not change significantly or increased, depending on harvest date and DPA treatment (Table 2).

Methanol is produced by apples (Flath et al., 1967) and its production increases during fruit ripening (Frenkel et al., 1998). In vitro, methanol is released from pectin by activity of pectin methylesterase (PME) (Wood and Siddiqui, 1971). PME activity increases during ripening of many different fruit (Kays, 1997) and the de-esterification of pectins has been suggested to be the primary source of methanol in fruit and other plant tissues (Nemecek-Marshall et al., 1995; Frenkel et al., 1998). In 'Fuji' apples, 3 kPa CO_2 during CA storage reduced the rate of softening, but methanol accumulation increased (Table 2), suggesting that methanol accumulation during high CO_2 storage may not result only from pectin de-esterification. Another possible source of methanol in plant tissues could be protein methyltransferase and protein repair processes (Nemecek-Marshall et al., 1995).

Responses to stress concentrations of O_2 and/or CO_2 in many fruit including apples, pears, avocado and strawberry, can include inhibition of pyruvate dehydrogenase, induction of alcohol dehydrogenase and pyruvate decarboxylase and subsequent accumulation of anaerobic products, such as ethanol, acetaldehyde and ethyl acetate (Ke et al., 1995). Accumulation of ethanol and acetaldehyde may be detrimental to normal metabolism leading to development of injury (Clijsters, 1965; Smagula et al., 1968; Ke et al., 1995; Choi, 1997). Factors that contributed to the incidence of CO_2-injury (e.g. high CO_2 partial pressure, duration of 20 kPa CO_2 exposure, harvest at advanced maturity, watercore severity) were also associated with the increase in ethanol, acetaldehyde and methanol. DPA treatment that prevented CO_2-injury also reduced the accumulation of these compounds. For individual fruit, there were significant positive correlations between the severity of CO_2-injury and concentrations of ethanol, acetaldehyde and methanol. However, the fact that apples stored in low O_2-low CO_2 storage had high concentrations of these compounds without developing injury (Table 2) indicates accumulation of ethanol, acetaldehyde and methanol is not the direct cause of CO_2-injury. Regardless of their role in the development of fruit injury, ethanol, acetaldehyde and methanol can reduce apple quality by contributing to off-flavor development.

In conclusion, 'Fuji' apples are susceptible to brown-heart (CO_2-injury) during CA storage. This is a CO_2-induced disorder as it was exacerbated by high CO_2 concentration in the storage atmosphere at 1 and/or 20 °C and in CA with low (1.5 kPa) or high (10 kPa) O_2 concentration. In 'Fuji' apples, CO_2-injury increases with late harvest and watercore severity. DPA prevented CO_2-injury and in some instances delayed ripening. Increases in internal ethanol, acetaldehyde and methanol during short-term high CO_2-storage were correlated with severity of CO_2-injury, however, these compounds accumulated in fruit stored in CA with 0.5 kPa O_2 without the development of injury.

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